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18.1 Introduction

Blood vessels play a significant role in the circulatory system [1]. The main function of blood vessels is transporting blood from the heart to the rest of the tissues and organs throughout the body and then bringing it back to the heart. Blood vessels include three main types: arteries, veins, and capillaries. Arteries transport blood from the heart to other parts of the human body, and veins carry blood from other parts of the body back to the heart; capillaries, which are the smallest blood vessels, are the connective parts between arteries and veins, serving a function of enabling substance exchange between the blood and the surrounding tissues [2]. There are also other types of blood vessels such as elastic arteries, distributing arteries, arterioles, venules, large collecting vessels (e.g., subclavian vein, jugular vein, renal vein, and iliac vein), and venae cavae, which can be still defined however, in general as arteries (for those transporting blood away from the heart) and veins (for those carrying blood toward the heart) [2].

The structures of blood vessels are crucial to their physiological functions [3,4]. As shown in Fig. 18.1, arteries and veins both have three layers, and the middle layer of arteries is much thicker than that of veins. The intima consists of squamous endothelial cells (ECs), which are intertwined with a polysaccharide intercellular matrix to form the lumen for blood transportation, whereas the veins contain valve structure to avoid backflow. Internal elastic lamina surrounds the exterior of the intima, which is composed by subendothelial tissues and elastic banding. The media is the middle layer in the vessels, where the elastic fibers, connective tissues, polysaccharides, and vascular smooth muscle cells (SMCs) are mainly located. The SMCs control the contraction and expansion of vessels, enabling the modulation of the hemodynamics. The adventitia contains nutrient capillaries and nerves to support the vessels. There is also an external elastic lamina between the media and adventitia. Capillaries are the connections between arteries and veins, composed of EC and connective tissues, which provide alternative pathways for transporting blood from arteries to veins.

Compared with the heart, blood vessels seldom actively pump blood to tissues. However, certain blood vessels can adjust inner diameters by the slight peristalsis from the contraction of the smooth muscle layer, which is controlled by the autonomic nervous system [5,6]. Vasoconstriction and vasodilation can also be regulated by vasoconstrictors (e.g., prostaglandins, vasopressin, angiotensin, and epinephrine) and vasodilator (e.g., nitric oxide). Oxygen bound to hemoglobin of red blood cells is one of the main nutrients transported by the blood vessel. Oxygen saturation of hemoglobin is typically in the range 95%–100% in all the arteries except the pulmonary

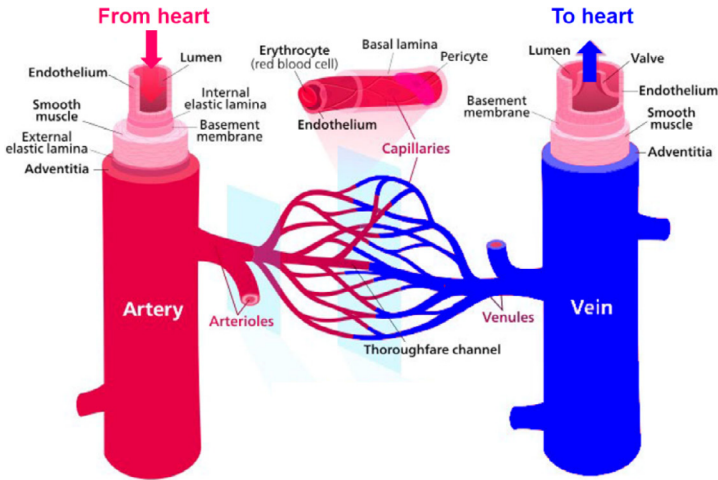


Fig. 18.1 Physiology structures of blood vessels including arteries, veins, and capillaries.

artery, whereas oxygen saturation of blood is about 75% in all the arteries except the pulmonary artery [2].

Blood vessels are significantly related to some common diseases. Angiogenesis in a tumor accelerates the progress of cancer by supporting the nutrition of the cancer cells [7]. The lipid lumps formed in the blood vessel wall can lead to atherosclerosis [8], which is the most common cardiovascular disease causing many deaths. Moreover, permeability of blood vessels increases during inflammation [9], and common hemorrhage induced by damage or in spontaneous cases is usually due to mechanical injury of the vascular endothelium. Besides, blockage of the blood vessels caused by internal and external factors (e.g., atherosclerotic plaque, embolized blood clot, or foreign body) probably leads to ischemia of tissue, sometimes even necrosis [10].

Tissue engineering has provided a promising strategy to repair and replace portions of tissues (e.g., bone, cartilage, blood vessels, and skin), using a combinatory approach including biology, medicine, materials, chemistry, and engineering [11–13]. Among the different tissue types, blood vessels are one of the most important yet challenging tissues to engineer, which are mainly fabricated by seeding proper cell types such as ECs and SMCs on suitable biomaterial substrates [14–16]. However, engineered blood vessels using conventional strategies based on scaffolds are usually produced using relatively sophisticated procedures, and cannot be easily applied to vessels with complex architectures. The recent advances in the three-dimensional (3D) bioprinting technology on the contrary, have provided unprecedented flexibility in engineering blood vessels with high resolution, strong fidelity, and high complexity [17]. In this chapter, vascular tissue engineering based on 3D extrusion bioprinting is discussed. We first introduce the different strategies for fabricating blood vessels, including sacrificial bioprinting, embedded bioprinting, hollow tube bioprinting, and microtissue bioprinting. Finally, we summarize with brief conclusions and future perspectives.

18.2 3D bioprinting strategies

Many strategies based on 3D bioprinting have been innovated over the decade for the fabrication of engineered blood vessels, where most commonly used include for example, sacrificial bioprinting, embedded bioprinting, hollow tube bioprinting, and microtissue bioprinting, among others.

Sacrificial bioprinting is probably the most established method to produce engineered blood vessels (Fig. 18.2A) [18–20]. In this approach, the sacrificial materials (e.g., agarose [21], carbohydrate glass [20], Pluronic F127 [19,22,23], and gelatin [24,25]) are usually used to fabricate the template channels within hydrogel matrices, which can be selectively removed or dissolved for subsequent seeding of vascular cells. The sacrificial bioprinting strategy can achieve the rapid fabrication of perfusable blood vessels in an efficient manner, potentially enabling the generation of complex vascular patterns. However, sacrificial bioprinting is limited in its ability to engineer spatially distributed blood vessel constructs due to the lack of support in the 3D volume, and therefore cannot satisfy some needs where the creation of sophisticated 3D vasculature is required.

To address the limitation associated with sacrificial bioprinting, embedded bioprinting has been proposed as a novel strategy to produce freeform 3D vascular constructs by taking advantage of the antigravity feature of the supporting hydrogels, which significantly improves the complexity of the blood vessels that can be bioprinted [26–28]. In a typical embedded bioprinting strategy (Fig. 18.2B), the shear-thinning bioink can be directly extruded into a self-healing supporting hydrogel matrix to build the arbitrarily shaped vessel-like structures.

Hollow tube bioprinting represents an additional strategy that can directly deposit hollow vascular structures in a single step, typically through the use of a bioprinting system in conjunction with the wet-spinning technique with a multilayer printhead (Fig. 18.2C) [29,30]. These bioprinted hollow tubes can transport fluids within the hollow interiors, which have similar characteristics with blood vessels. These hollow blood vessel-like structures can be fabricated by first bioprinting the tubes followed by culturing vascular cells or by directly bioprinting vascular cell-laden bioinks into the hollow structures.

Besides, other bioprinting strategies have also been developed to fabricate engineered blood vessels such as the use of spheroids and microtissues as the bioinks for printing, which take the spheroid or microtissues as the basic building units to enable

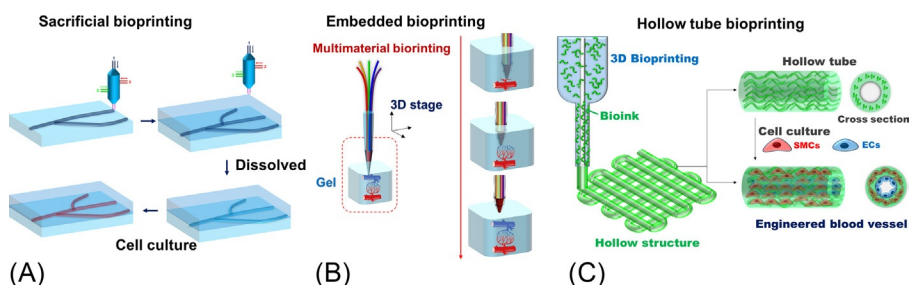


Fig. 18.2 Main types of 3D bioprinting strategies for engineering blood vessels: (A) sacrificial bioprinting, (B) embedded bioprinting, and (C) hollow tube bioprinting.

engineering of blood vessels at a relatively large scale than other strategies mentioned above [31]. All these bioprinting strategies will be separately discussed in details in the following sections.

18.3 Sacrificial bioprinting for vascular engineering

The common strategy of sacrificial bioprinting is based on the employment of sacrificial material to fabricate a sacrificial template, and a hydrogel matrix will be subsequently cast or bioprinted surrounding the template. Finally, this sacrificial template can be removed by mechanical pulling or dissolution. As such, the well-defined microchannels will be formed for vascular engineering. As aforementioned, a variety of biomaterials can be used as the sacrificial templates such as agarose [21], gelatin [24,25], Pluronic F127 [19,22,23], and carbohydrate glass [20], among others [32,33].

The Khademhosseini group reported a sacrificial bioprinting strategy based on agarose microfibers to achieve vascular structures via mechanical removal [21]. As shown in Fig. 18.3A, agarose was used as a sacrificial material, and the agarose microfibers were bioprinted in predefined vessel-like patterns. The gelatin methacryloyl (GelMA) hydrogel was cast over the bioprinted agarose microfibrillar templates. The bioprinted agarose microfibers were subsequently removed by manual pulling or using mild vacuum from the photo-cross-linked GelMA hydrogels. The perfusable microchannels were formed without an additional dissolution step, and GelMA hydrogel containing intrinsic cell-binding sites ensured strong attachment of human umbilical vein endothelial cells (HUVECs). Taking advantage of this sacrificial strategy, various vessel-like structures could be rapidly generated (Fig. 18.3B).

To improve further the biocompatibility of bioprinted vascular structure, Dai and colleagues developed a collagen-based vascular construct [24], which adopted a hydrogel of endothelial cell-laden gelatin as the sacrificial material for bioprinting at a lower temperature (Fig. 18.3C). Following a subsequent culture at 37°C in an incubator, the endothelial cells would attach to the surface of the microchannels as the gelatin liquefies and dissolves out over time, forming the engineered vessels presenting vascular morphology and barrier functions (Fig. 18.3D).

Lewis and coworkers developed a cell-laden, vascularized tissues with a thickness of more than 1 cm, including HUVECs, human mesenchymal stem cells (hMSCs), and human neonatal dermal fibroblasts (HNDFs) embedded in extracellular matrix materials, where high cell viability could be maintained for more than 6 weeks in a perfusable chip (Fig. 18.3E) [19]. These thick vascularized tissues were fabricated by sacrificial bioprinting using a composite fugitive bioink. The bioink containing Pluronic and thrombin were used to bioprint the template in a vascular pattern, where the cell-laden hydrogel containing gelatin and fibrinogen were used to surround the template to form the thick tissue. The fugitive bioink was then liquefied and removed to generate a large vascular network, which could be further endothelialized and perfused to achieve long-term survival of the thick tissue using a perfusion system (Fig. 18.3F–M).

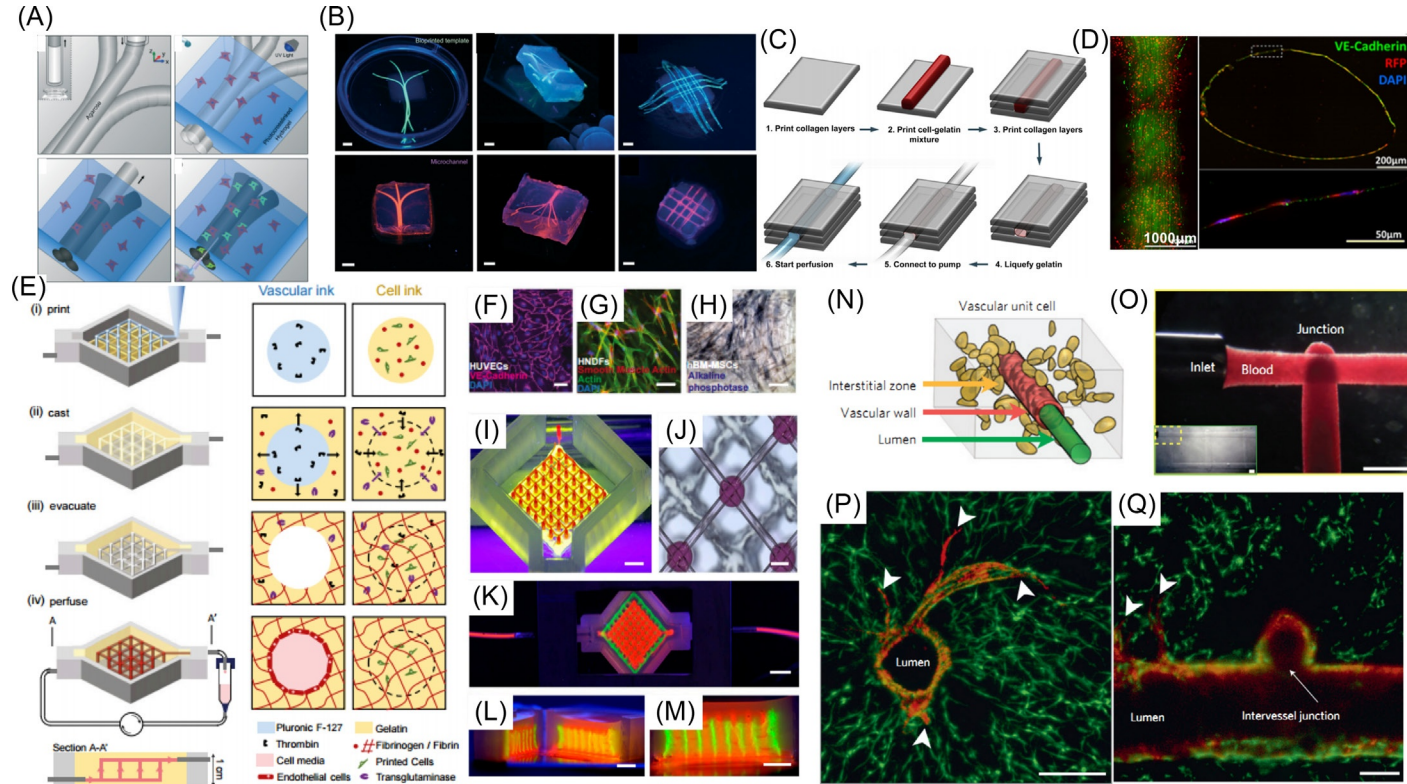


Fig. 18.3 See figure legend in next page

Similarly, the Chen Group reported the use of a network of rigid 3D filaments as the sacrificial template, printed with carbohydrate glass [20]. The formed vascular structure could be lined with endothelial cells, which included the lumen, the endothelialized vascular wall, the matrix, and cell-based interstitial zone (Fig. 18.3N). The vascular structure supported the pulsatile blood flow at high pressure (Fig. 18.3O). The endothelial cells formed patterned vasculature and intervessel junctions (Fig. 18.3P and Q).

These sacrificial bioprinting strategies made the construction of the vascular network, the endothelialization, and the extravascular tissue independent, potentially compatible with various types of cells and extracellular matrices for engineering various types of tissues. However, the sacrificial materials have been usually associated with different degrees of cytotoxicity originating from template dissolution. For example, the bioprinted sacrificial carbohydrate glass templates required coating with poly(lactide-*co*-glycolide) (PLGA) to prevent osmotic damage to cells enclosed

Fig. 18.3 continued Sacrificial bioprinting for vascular engineering. (A) Schematic of agarose template fiber-based bioprinting. The agarose template is bioprinted by a bioprinter with a piston fitted inside a glass capillary aspirating at predefined locations after gelation, which is subsequently cast over by a hydrogel. Fully perfusable microchannels were formed after the agarose template is removed from the photo-cross-linked gel. (B) Photographs of the bioprinted templates (green) enclosed in GelMA hydrogels and the respective microchannels perfused with a fluorescent microbead suspension (pink) (Scale bars: 3 mm). (C) Construction processes of the vascular channel using cell-gelatin mixture as a sacrificial material. (D) Fluorescence images of the flow of green beads and bioprinted endothelial cells on Day 1 under dynamic flow culture. After 5 days of dynamic culture, it could be found from a cross section that endothelial cells formed a monolayer along the channel surface. (E) Thick vascularized structure is bioprinted in a perfusion chip by fugitive bioink and cell-laden bioink. The extracellular matrix (ECM) material is then cast over the bioprinted vascular structure. The sacrificial bioink is then liquefied and evacuated. The vascular structure is subsequently endothelialized and perfused. (F–H) HUVECs, HNFs, and hMSCs growing in the bioprinted matrix (Scale bar: 50 μ m). (I) Photographs of sacrificial structure (red) on chip (Scale bar: 2 mm). (J) Top view image of sacrificial structure (red) and cell inks (green) (Scale bar: 50 μ m). (K–M) Image of a 3D bioprinted tissue with vascular unit cell in a perfusion chamber (K) and their cross sections (L and M) (Scale bars: 5 mm). (N) Schematic of vascular unit cell including the lumen, the endothelialized vascular wall, and the matrix and cell-based interstitial zone. (O) Vascular channels and intervessel junctions support pulsatile flow of human blood. (P) Endothelial cells formed patterned vasculature at the arrow position. (Q) Intervessel junctions near the larger vessels. (A–B) Reproduced with permission from Bertassoni LE, Cecconi M, Manoharan V, Nikkha M, Hjortnaes J, Cristino AL, et al. Hydrogel bioprinted microchannel networks for vascularization of tissue engineering constructs. *Lab Chip* 2014;14(13):2202–2211. Copyright 2014 Royal Society of Chemistry; (C–D) Reproduced with permission from Lee VK, Kim DY, Ngo H, Lee Y, Seo S-S, et al. Creating perfused functional vascular channels using 3D bio-printing technology. *Biomaterials* 2014;35(28):8092–8102. Copyright 2014 Elsevier; (E–M) Reproduced with permission from Kolesky DB, Homan KA, Skylar-Scott MA, Lewis JA. Three-dimensional bioprinting of thick vascularized tissues. *Proc Natl Acad Sci U S A* 2016;113(12):3179–3184. Copyright 2016 National Academy of Sciences; (N–Q) Reproduced with permission from Miller JS, Stevens KR, Yang MT, Baker BM, Nguyen D-HT, Cohen DM, et al. Rapid casting of patterned vascular networks for perfusable engineered three-dimensional tissues. *Nat Mater* 2012;11(9):768–774. Copyright 2012 Nature Publishing Group.

inside the hydrogel during the dissolution process [20]. However, highly concentrated Pluronic has shown cytotoxic effects for cells embedded within the surrounding matrices [22]. Therefore, it is of paramount importance to further optimize the formulations of the sacrificial bioinks to improve biocompatibility and expand the applicability of sacrificial bioprinting in vascular engineering.

18.4 Embedded bioprinting for vascular engineering

Conventional extrusion bioprinting can deposit structures in a layer-by-layer manner, which, however, is difficult to achieve freeform structures due to lack of support in the volumetric space, precluding the fabrication of complex blood vessels. To address such a challenge, embedded bioprinting recently emerged to provide a new strategy to bioprint freeform patterns in 3D space at high resolution.

Burdick and colleagues initially developed the embedded bioprinting that allowed direct extrusion of shear-thinning hydrogel bioinks into self-healing support hydrogels [26]. They used the supramolecular hydrogels based on conjugation of adamantane (Ad) and β -cyclodextrin (CD), respectively, to hyaluronic acid (HA), which showed good biocompatibility [34] (Fig. 18.4A). Specifically, Ad-HA and CD-HA could rapidly form guest-host complexes immediately after mixing (Fig. 18.4B). It is therefore possible that this guest-host hydrogel system be directly writable due to their noncovalent and reversible bonds that could be disrupted by a physical stimulus (e.g., shear stress) and reform rapidly after the removal of the stimulus [35]. These properties qualified their use for embedded bioprinting, where the support matrix could accommodate the bioprinted material to maintain the structure through self-healing [36,37]. Consequently, 3D filaments could be deposited within the support hydrogel, and their diameters and patterns might also be easily controlled by the movement and size of needles (Fig. 18.4C–F).

The Feinberg Group further reported a similar strategy by applying the soft protein and polysaccharide hydrogels to bioprint the complex 3D biological structures in freeform, termed *freeform reversible embedding of suspended hydrogels* (FRESH) [27]. FRESH-enabled bioprinting of complex structures in a thermoreversible support bath using computer-aided design (CAD) models derived from optical computed tomography (OCT) and magnetic resonance imaging (MRI) data at a resolution of $\sim 200\ \mu\text{m}$ (Fig. 18.4G and H). Alternatively, microparticles-based soft granular hydrogels also appear to be efficient support materials for embedded bioprinting of arbitrary structures due to their shear-thinning behaviors [38–40]. Granular gels made from soft microparticles can smoothly transit between the solid and fluid states, making them ideal for holding complex and high-resolution bioprinted structures. Accordingly, the Angelini Group bioprinted complex 3D objects consisting of branching vessel-like networks using hydrogels, colloids, silicones, and living cells [28]. As shown in Fig. 18.4K, as a fine injection tip extruded the bioink in a predefined spatial path, the support granular gel could rapidly solidify after temporarily fluidizing locally, leading to stabilization of the bioprinted pattern at the injection site. Bioprinted freeform structures could be cross-linked and removed from the granular gel. This approach provides a versatile way to engineer diverse vascular structures with varying diameters and branches (Fig. 18.4L).

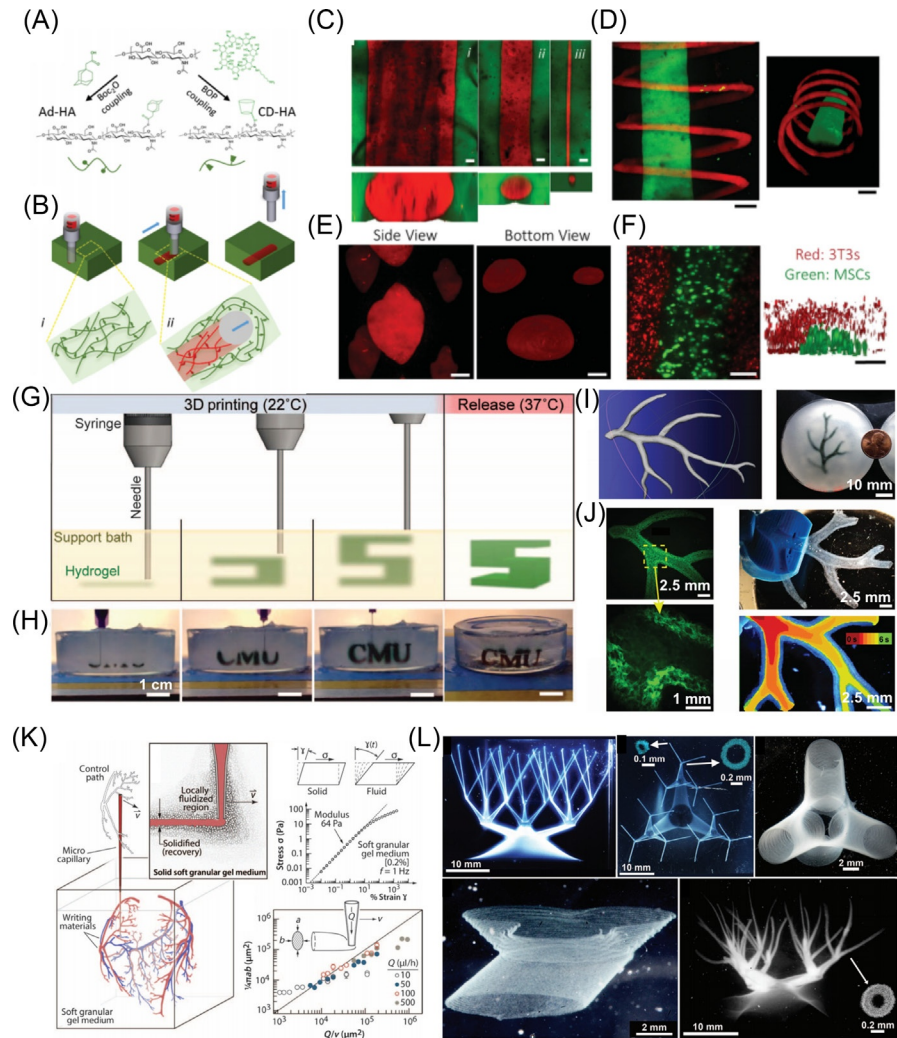


Fig. 18.4 Embedded bioprinting for vascular engineering. (A) Conjugation of Ad and β -CD to HA. (B) Schematics of embedded bioprinting by injecting a supramolecular ink (red) into a supramolecular support gel (green) from the original network (i) to partial cross-link pattern (ii). (C) Rhodamine-labeled filaments extruded into a fluorescein-labeled support gel by needles with different diameters of 20 G (i), 27 G (ii), and 34 G (iii). (Scale bars: 100 μ m). (D) A filament of a fluorescein-labeled ink with a continuous spiral pattern (Scale bars: 200 μ m). (E) Discrete pockets of a rhodamine-labeled ink printed into an unlabeled support gel (Scale bars: 200 μ m). (F) hMSCs (green) bioprinted within a bioink into a support gel containing NIH/3T3 fibroblasts (red) (Scale bars: 200 μ m). (G) Schematic of the FRESH processes, showing the bioprinted and cross-linked hydrogel (green) in the gelatin support bath (yellow). The 3D structure is fabricated layer by layer and the support gelatin melted to release a bioprinted structure by heating at 37°C. (H) Alginate "CMU" were built by FRESH bioprinted in the font of Times New Roman. The letters are still not deforming when the gelatin support

18.5 Hollow tube bioprinting for vascular engineering

Although the sacrificial and embedded bioprinting strategies enable the generation of vascular structures, they generally involve complicated multistep procedures. To this end, hollow tube bioprinting provides an alternative to build vessel-like channel, better imitating the anatomy and physiology of blood vessels [29,41,42,30].

Based on alginate, Ozbolat and colleagues developed the technique to bioprint tubular microchannels mimicking the blood vessels, which could be constantly perfused to provide nutrients for encapsulated cells [29]. For this hollow tube bioprinting, a coaxial nozzle was designed, where alginate bioink was extruded from the sheath flow whereas the CaCl_2 solution was delivered from the interior (Fig. 18.5A–E). Vessel-like hollow microfibers were formed on cross-linking of the sheath alginate bioink by Ca^{2+} . The cell viability could be assured by optimization of the bioprinting conditions such as the geometry of the coaxial nozzle as well as the concentrations of the bioinks and the cross-linking agent. Due to the shear stress, the encapsulated cells would experience certain damage during the bioprinting process (Fig. 18.5F); however, the cells could gradually recover during subsequent culture (Fig. 18.5G), where their functionality was also validated by evaluating the tissue-specific biomarker expression and extracellular matrix production.

The bioink formulation is another critical parameter in hollow tube bioprinting especially when improved functions of the bioprinted cells are required. Hydrogel materials such as gelatin, collagen, hyaluronic acid, alginate, and poly(ethylene glycol) (PEG),

Fig. 18.4 continued melts the change in optical, fluidic properties. (I) A section of human right coronary arterial tree from 3D MRI is translated into machine code for FRESH bioprinting, and was bioprinted with alginate (black) in the gelatin support bath. (J) The bioprinted arterial trees showing the vessel wall, well-formed lumen, and multiple bifurcations under fluorescent alginate (green). The engineered vessel was validated to be well sealed by perfusion. (K) Granular gel as an alternative embedded bioprinting support materials. A capillary tip extruded out material into the granular gel to form a complex pattern. The granular gel locally fluidizes and then rapidly solidifies when the tip moves across it, so the complex structures can be built without solidifying or cross-linking to support themselves. Stress-strain measurements presented a shear modulus of 64 Pa and a yield stress of 9 Pa for 0.2% (w/v) Carbopol gel. The cross-sectional area of embedded bioprinting showed nearly ideal behavior $Q = \frac{1}{4}\pi r^2 v$ over a wide range of tip speeds, v , and flow rates, Q . (L) Diverse branched vascular networks based on the granular gel support materials. A continuous hollow vessel network with several orders of magnitude in diameter and aspect ratio. (A–F) Reproduced with permission from Highley CB, Rodell CB, Burdick JA. Direct 3D printing of shear-thinning hydrogels into self-healing hydrogels. *Adv Mater* 2015;27(34):5075–5079. Copyright 2015 John Wiley & Sons, Inc; (G–J) Reproduced with permission from Bhattacharjee T, Zehnder SM, Rowe KG, Jain S, Nixon RM, Sawyer WG, et al. Writing in the granular gel medium. *Sci Adv* 2015;1(8):e1500655. Copyright 2015 American Association for the Advancement of Science; (K–L) Reproduced with permission from Hinton TJ, Jallerat Q, Palchesko RN, Park JH, Grodzicki MS, Shue H-J, et al. Three-dimensional printing of complex biological structures by freeform reversible embedding of suspended hydrogels. *Sci Adv* 2015;1(9):e1500758. Copyright 2015 American Association for the Advancement of Science.

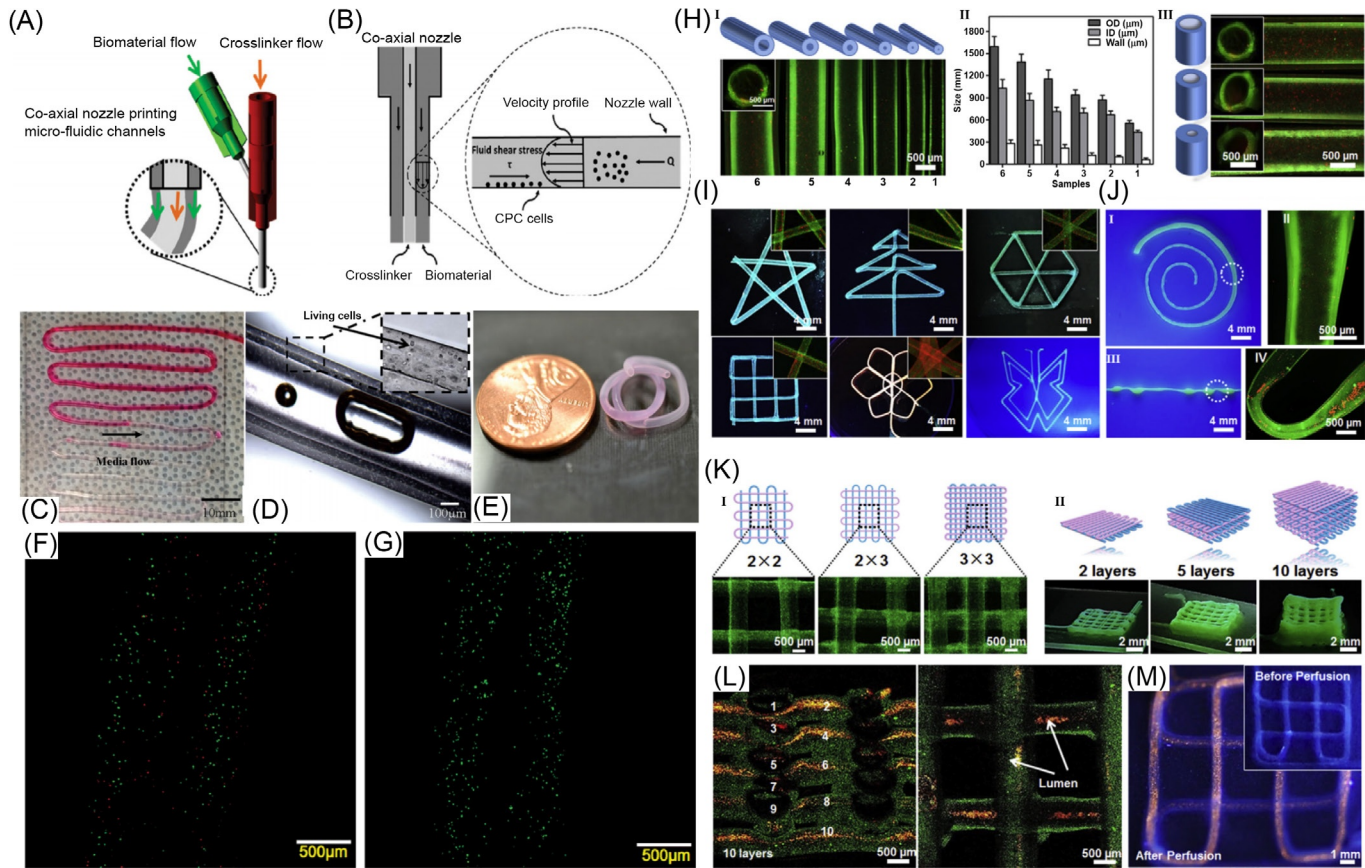


Fig. 18.5 Hollow tube bioprinting for vascular engineering: (A) schematic of coaxial nozzle for hollow tube bioprinting, (B) shear stress generated in the coaxial nozzle system, (C) cell-laden tubular channels were bioprinted in the form of zigzag with medium perfusion,

have been used as bioinks based on their biocompatibility and sufficient viscosity for bioprinting [34,43,44]. Nevertheless, none of them alone offers suitable properties for direct bioprinting of perfusable hollow tubes. The Khademhosseini Group recently reported a hollow tube bioprinting strategy by engineering a blend bioink containing alginate, GelMA, and 4-arm PEG-tetra-acrylate (PEGTA) [30], which not only possessed suitable rheological properties for use as the bioink, but also had sufficient mechanical strength and biocompatibility to support the functionality of the bioprinted hollow tubes. The alginate component in the blend bioink could be cross-linked by the delivered Ca^{2+} in both the core and the sheath to maintain the initial shape of extruded hollow tubes. Following bioprinting, the morphologies of the hollow tubes were chemically fixed by photo-cross-linking of the GelMA and PEGTA components. This blend bioink-based hollow tube bioprinting could easily achieve control over the size and pattern of the generated hollow tubes (Fig. 18.5H–M). The PEGTA molecules in the bioink led to the formation of a loose network with large pores to support both the strong mechanics of the blood vessels and the proliferation/stretching of the embedded vascular cells, whereas the biocompatible nature of GelMA further ensured functionality of the cells.

18.6 Scaffold-free multicellular spheroid bioprinting for vascular engineering

For vascular engineering, the choice of matrix materials plays a significant role. The immunogenicity, degradation rate, toxicity of degradation products, host inflammatory responses, and mechanical properties should all be considered. These potential problems associated with biomaterials may affect the applications of engineered vascular constructs, and may directly interfere with their biological functions in the long term,

Fig. 18.5 continued (D) images of vessel-like hollow structure and cell encapsulation in the channel wall, (E) cell-laden hollow tube showing promising mechanical and structural integrity after a 1-week culture, (F) confocal image for live/dead staining of at 12-h postprinting, massive cell death (red fluorescent) was observed all over the printed structure, (G) after a 72-h incubation, a few dead cells were scattered among increased live cells, (H) bioprinted hollow tube with different outer diameters (I) and quantification data (II), with same outer diameters and different inner diameters (III), (I) different bioprinted perfusable tubes with various shapes, (J) hollow tube with a gradually increasing size and periodically varying sizes, (K) images of the bioprinted hollow tube-based grids with different aspect ratios and numbers of layers, (L) confocal images showing a uniform 3D structure with 10 layers of bioprinted hollow tubes, which were perfused with red fluorescent microbeads, and (M) fluorescence images before (inset) and after the injection with red fluorescent microbeads into the lumen of the continuous bioprinted hollow tube.

(A–G) Reproduced with permission from Yu Y, Zhang Y, Martin JA, Ozbolat IT. Evaluation of cell viability and functionality in vessel-like bioprintable cell-laden tubular channels. *J Biomech Eng* 2013;135(9):091011. Copyright 2013 American Society of Mechanical Engineers; (H–M) Reproduced with permission from Jia W, Gungor-Ozkerim PS, Zhang YS, Yue K, Zhu K, Liu W, et al. Direct 3D bioprinting of perfusable vascular constructs using a blend bioink. *Biomaterials* 2016;106:58–68. Copyright 2016 Elsevier.

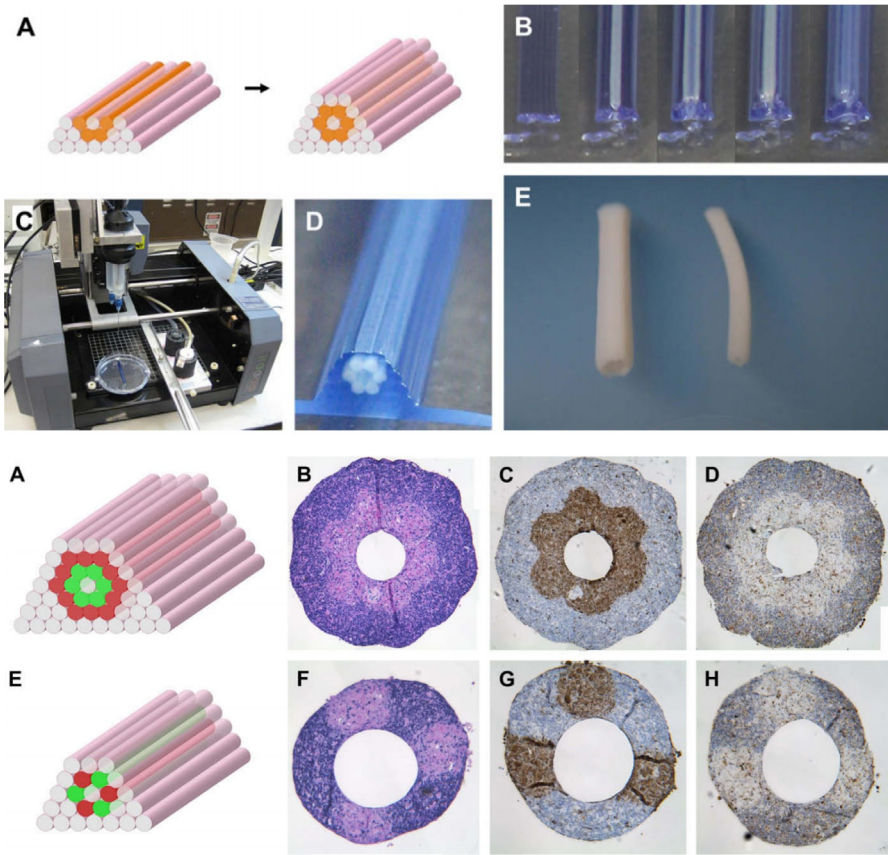


Fig. 18.6 Fusion patterns of multicellular spheroids and cellular cylinders for vascular engineering. (A) Schematics of deposition of the smallest diameter tube by agarose rods (pink) and multicellular spheroids (orange) with same diameters. (B) Different complex tubular structures. (C) Branched structure. (D) HSF spheroids bioprinted per template in (A). (E) Fusion pattern labeled red and green CHO spheroids in a tube after 7 days. (F) Branched structure based on HSF spheroids with 1.2 and 0.9 mm of diameters. (G) The fused branched structure after 6 days. (H, I) Design template by deposition of agarose cylinders (blue) and multicellular SMC cylinders. (J) Bioprinter with two printheads. (K) Bioprinted construct. (L) Engineered pig SMC tubes after 3 days of postprinted fusion (left: 2.5 mm OD; right: 1.5 mm OD). (M, Q) A double-layered vascular wall. HUVSMC (green) and HSF (red) multicellular cylinders were assembled per specific patterns. (N, R) Hematoxylin and eosin, (O, S) smooth muscle α -actin (brown), and (P, T) caspase-3 (brown) staining showing the results of the respective structures in (M) and (Q) after 3 days of fusion.

(A–T) Reproduced with permission from Norotte C, Marga FS, Niklason LE, Forgacs G. Scaffold-free vascular tissue engineering using bioprinting. *Biomaterials* 2009;30(30):5910–5917. Copyright 2009 Elsevier.

especially when clinical translation is the immediate goal [45]. Therefore, scaffold-free bioprinting has emerged as another strategy to fabricate vascular structures.

In one example, Forgacs and colleagues introduced a rapid prototyping technique based on 3D-automated deposition of multicellular spheroids or cylinders by a bio-printer (Fig. 18.6A–C, H, M, Q) [31]. The 3D tissue structures were formed through the postbioprinting fusion of the multicellular spheroids or cylinders (Fig. 18.6E, G, N–P, R–T), which is similar to the self-assembly phenomenon observed during early morphogenesis [46]. Delivery of multicellular spheroids by bioprinting was rapid and accurate, and assured maximal cell density leading to minimal cell damage. A fully biological self-assembly approach was thus developed by a rapid scaffold-free prototyping bioprinting for fabrication of blood vessels of various sizes (OD: 0.9–2.5 mm) (Fig. 18.6D–G). Multiple types of cells such as SMCs, human skin fibroblasts (HSF), Chinese Hamster Ovary (CHO) cells, and human umbilical vein smooth muscle cells (HUVSMCs) were integrated into discrete units. The multicellular spheroids or cylinders had controllable diameters (300–500 μm), and agarose rods were used as a template in this bioprinting strategy. This unique scaffold-free bioprinting strategy enabled the engineering of vascular structures without the use of any biomaterials and can be potentially extended to fabricate blood vessels at clinically relevant scales.

18.7 Conclusion

In this chapter, we focused on the discussions of various 3D bioprinting strategies for vascular engineering, including sacrificial bioprinting, embedded bioprinting, hollow tube bioprinting, and scaffold-free multicellular spheroid-based bioprinting. Key examples of each bioprinting strategy were illustrated. It is anticipated that further improvements of the bioink formulations would play a crucial role in assuring the stability of the bioprinted vascular structures as well as the maintenance of good cell viability and functionality. A combination of different strategies might also generate additional advantages for vascular bioprinting, such as deposition of multiscale patterns. We believe that 3D extrusion bioprinting will become a promising and powerful approach in engineering sophisticated and functional blood vessels for widespread applications in both in vivo transplantation and in vitro drug screening.

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